

Jeffrey S. Ross, MD
Department of Pathology Mail Code 81
Albany Medical College
47 New Scotland Avenue
Albany, New York 12208

(518) 262-5471
(518) 262-8092

Exhibit E

PROSTATE SPECIFIC MEMBRANE ANTIGEN (PSMA) EXPRESSION IN NON-PROSTATE CANCERS

JS Ross, K Gray, S Beddah, D Schenkein, I Webb, G Gray, N Leschly, R Mosher, R Meyer, J Deeds. Albany Medical College, Albany NY and Millennium Pharmaceuticals, Inc., Cambridge, MA

Background: PSMA, a transmembrane folate hydrolase, is expressed at low levels in normal prostate epithelium and is significantly overexpressed in prostate cancer (PCA) and the neovasculature of various non-PCAs. Non-PCA expression of PSMA is currently being pursued as a target for diagnostic imaging and anti-cancer antibody therapeutics.

Design: PSMA expression was measured on a series of fresh frozen non-PCA malignancies by transcriptional profiling (TP) using cDNA microarrays on nylon membranes, RT-PCR (Taqman), in situ hybridization, western blotting, dual co-localization immunofluorescence (IF) and immunohistochemistry (IHC) both before and after laser capture microdissection (LCM).

Results: PSMA mRNA expression by ISH was localized to the neo-vasculature in 55% of a series of breast, colon, lung and ovarian cancers. PSMA mRNA expression measured by Taqman™ RT-PCR was localized to the endothelium of the tumor vessels after microdissection. Using IHC with the J-591 antibody to the external domain of PSMA on frozen sections, 40% of the same carcinomas were positive for PSMA immunoreactivity of the tumor vasculature. Dual IF studies using antibodies to PSMA and CD31 (PECAM-1) localized PSMA expression to the endothelium of neovasculature in carcinomas of the breast, colon, lung and ovary, in Wilms' tumors and neuroblastomas, but not in the tumor vessels of PCAs.

Conclusion: These molecular studies confirm that PSMA expression is highly associated with the neo-vasculature of many non-PCAs and co-localizes with endothelial cell markers. Further studies of PSMA in non-PCA as a target for both diagnostic imaging and anti-cancer antibody-based therapies appear warranted.

BEST AVAILABLE COPY